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Comparison of Airborne Asbestos Levels Determined by Transmission Electron Microscopy (TEM) Using Direct and Indirect Transfer Techniques

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FINAL REPORT

COMPARISON OF AIRBORNE ASBESTOS LEVELS DETERMINED BY TRANSMISSION ELECTRON MICROSCOPY (TEM) USING DIRECT AND INDIRECT TRANSFER TECHNIQUES

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AUTHORS AND CONTRIBUTORS

This report was prepared by Jean Chesson of Chesson Consulting, Inc. under subcontract to Battelle. Jeff Hatfield of Battelle prepared an earlier draft of the Study 1 (EPA 1988) results. The R.J. Lee Group, Inc., Monroeville, PA performed the laboratory analysis of samples from Study 1 as part of the study reported in EPA (1988).

The EPA work assignment manager was Brad Schultz. Substantial contributions were also made by Cindy Stroup, Betsy Dutrow, and Joe Breen of the Exposure Evaluation Division in the EPA Office of Toxic Substances.

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EXECUTIVE SUMMARY

Background

Transmission electron microscopy (TEM) is the preferred analytical method for measuring asbestos concentrations in ambient atmospheres. The absence of a standard protocol for TEM analysis and the discovery and refinement of new techniques have resulted in a variety of procedures which may not necessarily provide comparable estimates of airborne asbestos concentration. An important difference between protocols is the use of direct and indirect transfer techniques. The direct transfer method was developed primarily to estimate structure concentration, whereas the indirect transfer method was developed primarily to estimate mass concentration. In a direct transfer the original filter is prepared for analysis with minimal disturbance of the particles upon it. In an indirect transfer, the particles are removed from the original filter and resuspended on a second filter prior to microscopic examination. Although the original spatial distribution of the particles is lost, indirect transfer is thought to provide greater control over analytical precision through improved distribution of materials over the surface of the filter.

Early TEM measurements of airborne asbestos used an indirect transfer method and expressed the results in terms of mass (ng/m^3). Fiber concentrations were not reported because it was thought that the indirect transfer technique might have broken up larger asbestos structures and artificially inflated the fiber count. The U.S. Environmental Protection Agency (EPA) has used the indirect transfer technique for many of its research programs, in part to overcome the problem of non-asbestos debris in some sampling situations, and in part because the type of filter most suited for direct transfer (polycarbonate) was thought to be more difficult to handle and transport in the field. However, improvements in the direct transfer technique applied to mixed cellulose filters have made direct transfer a feasible option.

Prior to carrying out a recent study of airborne asbestos levels in public buildings (USEPA 1988), EPA convened a meeting of microscopists and other asbestos measurement experts to determine the most appropriate analytical protocol. A direct transfer method using mixed cellulose ester filters was selected. A similar TEM protocol was later specified under the Asbestos Hazard Emergency Response Act (AHERA) to determine when an asbestos work site is sufficiently clean for the containment barriers to be removed.

To investigate the relationship between airborne asbestos levels measured by the two transfer techniques and possibly provide a basis for comparison with earlier studies based on indirect transfer, a subset of the samples collected in the 1988 EPA study were reanalyzed using an indirect transfer method. This document reports the results of the EPA analysis and extends the discussion to include data from six other studies.

Results and Conclusions

The investigation confirmed the generally held opinion that the direct and indirect transfer methods provide different estimates of airborne asbestos concentration. There is insufficient information, however, to determine the mechanisms responsible for the difference and thereby recommend one method over the other. The specific conclusions are listed below followed by recommendations for further research.

- TEM analysis of air samples using indirect transfer methods tends to provide estimates of total airborne asbestos structure concentration that are higher than those obtained using direct transfer methods. This conclusion is consistent with general opinion and implies that airborne asbestos levels estimated by one method are not directly comparable to those estimated by the other.

  Evidence. A review of available data (seven studies) revealed this relationship in every study despite variations in sampling, analytical, and counting protocols.

- There is no single factor that can be applied to convert measurements made using an indirect transfer method to a value that is comparable with measurements made using a direct transfer method. The quantitative relationship between estimates obtained by the two transfer methods is expected to depend on sampling and analytical protocols as well as the nature of the asbestos structures in the air.

  Evidence. In the studies considered here, measurements made by the indirect transfer method were 3.8 times to 1,700 times higher than measurements made by the direct transfer method. The highest value of 1,700 was estimated from a set of 45 samples collected in a school district. The lowest value of 3.8 was obtained in an interlaboratory study of 12 samples of amphibole.

- **Provided a single method is applied consistently, the choice of method is not as critical when measurements are to be used only for comparative purposes (for example, comparison of airborne asbestos levels inside and outside an abatement site). When measurements are to be interpreted in relation to a fixed standard, the choice of method is more important.**

  Evidence. Both methods appear to detect changes in airborne asbestos concentrations. Although the relationship between the two methods is not strong, higher concentrations determined from one method tend to correspond to higher levels obtained by the other. A statistically significant relationship of this type was found between measurements made by the two transfer methods in all seven studies. In a study designed to compare indoor and outdoor airborne asbestos levels, the same trend was revealed by both methods.

- **Based on data from the studies considered in this report, it seems unlikely that the larger airborne asbestos concentrations estimated by the indirect transfer method can be explained solely by breakdown of large asbestos structures into smaller components. Alternative hypotheses involving interference by debris and association of unattached structures may also be important.**

  Evidence. In the two studies for which data are readily available, the indirect transfer method counted more structures than the direct transfer method in all size categories. One would expect to count fewer large structures with the indirect transfer method if larger asbestos structures were being broken down into smaller ones.

Additional Research

The information needed to select the appropriate protocol for a given situation could be obtained with a relatively modest research program. A series of studies is suggested to:

- Further investigate structure size distributions for direct and indirect TEM preparations in order to distinguish among alternative hypotheses and thereby determine which method more accurately reflects biologically meaningful airborne asbestos concentrations; and

- Compare the spatial distribution of asbestos structures on samples prepared by direct and indirect transfer methods in order to characterize the precision of each method.

II. CONCLUSIONS AND RECOMMENDATIONS

The results from the recent EPA study (USEPA 1988), together with a review of six other studies in the literature (Tuckfield et al 1988, Lee 1987 (two data sets), Burdett 1985a, Chatfield 1986, and Cook and Marklund 1982) lead to the following conclusions:

- **TEM analysis of air samples using indirect transfer methods tends to provide estimates of total airborne asbestos structure concentration that are higher than those obtained using direct transfer methods.** This conclusion is consistent with general opinion and implies that airborne asbestos levels estimated by one method are not directly comparable to those estimated by the other.

  Evidence. A review of available data (seven studies) revealed this relationship in every study despite variations in sampling, analytical, and counting protocols.

- **There is no single factor that can be applied to convert measurements made using an indirect transfer method to a value that is comparable with measurements made using a direct transfer method.** The quantitative relationship between estimates obtained by the two transfer methods is expected to depend on sampling and analytical protocols as well as the nature of the asbestos structures in the air.

  Evidence. In the studies considered here, measurements made by the indirect transfer method were 3.8 times to 1,700 times higher than measurements made by the direct transfer method. The highest value of 1,700 was estimated from a set of 45 samples collected in a school district. The lowest value of 3.8 was obtained in an interlaboratory study of 12 samples of amphibole.

- **Provided a single method is applied consistently, the choice of method is not as critical when measurements are to be used only for comparative purposes (for example, comparison of airborne asbestos levels inside and outside an abatement site). When measurements are to be interpreted in relation to a fixed standard, the choice of method is more important.**

  Evidence. Both methods appear to detect changes in airborne asbestos concentrations. Although the relationship between the two methods is not strong, higher concentrations determined from one method tend to correspond to higher levels obtained by the other. A statistically significant relationship was found between measurements made by the two transfer methods in all seven studies. In a study designed

to compare indoor and outdoor airborne asbestos levels, the same trend was revealed by both methods.

- **Based on data from the studies considered in this report, it seems unlikely that the larger airborne asbestos concentrations estimated by the indirect transfer method can be explained solely by breakdown of large asbestos structures into smaller components.** Alternative hypotheses involving interference by debris and association of unattached structures may also be important.

  Evidence. In the two studies for which data are readily available, the indirect transfer method counted more structures than the direct transfer method in all size categories. One would expect to count fewer large structures with the indirect transfer method if larger asbestos structures were being broken down into smaller ones.

Selection of an appropriate protocol in a given situation involves consideration of bias (systematic error) and precision (random error). The conclusions above, combined with opinions expressed by microscopists, indicate that the indirect and direct transfer methods differ with respect to bias and precision, but there is insufficient information to recommend one method over the other. The necessary information could be obtained with a relatively modest research program involving the analysis of existing data and experiments designed specifically for this purpose. It is recommended that studies be performed to:

- Further investigate structure size distributions for direct and indirect TEM preparations in order to distinguish among alternative hypotheses and thereby determine which method more accurately reflects biologically meaningful airborne asbestos concentrations; and

- Compare the spatial distribution of asbestos structures on samples prepared by direct and indirect transfer methods in order to characterize this component of precision.

These recommendations are discussed in more detail in Section VI.

VI. DISCUSSION

An analytic method should be sufficiently accurate for its intended purpose. Accuracy has two components: bias and precision. Bias refers to a systematic deviation of the measured value from the true value of the quantity being measured. In this case the objective is to characterize exposure in a biologically meaningful way, that is, in terms of the number and type of structures that are inhaled. Precision refers to the uncertainty associated with repeated measurements of the same quantity. The direct transfer method is often characterized as being less biased than the indirect transfer method, whereas the indirect transfer method is considered more precise by some researchers. Neither of these claims is supported by extensive data. Bias and precision are discussed in turn below, together with suggestions for further research that could assist in selecting the appropriate analytical method for a given situation.

A. Bias

Bias must be considered within the context of the application. If measurements are to be used in a comparative manner (e.g., comparing airborne asbestos levels inside and outside a building), a bias that applies equally to both sets of measurements may not affect the comparison. If, however, the objective is to measure exposure in order to assess risk, a bias may have a significant impact on the interpretation of the data. Although the details are controversial, it is thought that the dimension of asbestos structures is important in determining the incidence of disease. Special attention should be devoted to minimizing bias with respect to asbestos structures that contribute most to disease incidence. (Note that the contribution is determined not only by relative potency of asbestos structures of different sizes, but also by their relative abundances.) An ideal measurement method would mimic the effect of respiration, etc. on complex structures (BCM) so that those that readily disintegrate would be represented by their individual components, while those that are firmly linked would be counted and sized as single structures.

The studies considered in this paper all support the generally accepted belief that airborne asbestos concentrations estimated by an indirect transfer method are larger than those estimated by a direct transfer method. Breakdown of larger structures during the ashing, sonication, and resuspension steps is assumed to be the main explanation for the difference. Fiber size information from Studies 1 and 5, however, does not provide strong support for this hypothesis. Although more small fibers are counted using an indirect transfer method, there is not a corresponding

decrease in the number of large fibers and BCM, nor in the size of the BCM.

Chatfield (1986) provides two additional hypotheses for the larger structure counts obtained with an indirect transfer method. First, with the direct transfer method, structures may be hidden by organic debris. (This hypothesis was also suggested by Sebastien et al, 1984.) The effect is likely to be greatest for small structures, but applies to structures of all sizes. During indirect transfer the debris is removed by ashing, thereby improving visibility and increasing the structure count. Second, with the direct transfer method, small structures loosely associated with larger structures (for example, touching but not bonded) are counted as a single structure. During indirect transfer, these structures are disassociated from the larger structures and are counted as individual structures.

All three mechanisms may play a role to a varying degree under different circumstances. Note that predictions depend on the size distribution of asbestos structures in the sampled air. When only small fibers are present, the breakdown hypothesis would predict little difference between direct and indirect preparations whereas the debris hypothesis would predict higher measurements with the indirect preparation. When the majority of structures are complex, the breakdown hypothesis would predict higher measurements with the indirect preparation whereas the association hypothesis would predict little difference.

Given that measurements by indirect TEM are generally higher than those by direct TEM, it is important to determine whether indirect measurements incorporate a positive bias (because, for example, the additional preparation artificially inflates the number of fibers) or the direct measurements incorporate a negative bias (because, for example, fibers are covered by debris). Fiber size data should be available for Studies 2, 3, and 4, and could be analyzed to distinguish between competing hypotheses. The number of structures counted, particularly those in the larger size categories, could limit the investigation. A designed experiment in which samples were prepared according to carefully specified protocols would provide more conclusive information. Experimental factors include preparation method, filter loading (low to high), and prevalence of complex structures.

B. Precision

Other considerations being equal, the method with the highest precision is preferable. For TEM analysis of airborne asbestos, the spatial distribution of asbestos structures on the surface of a filter is important in determining precision. Only a tiny fraction of the original filter area is examined with the electron microscope. It is assumed that the area is

representative of the entire filter surface in order to estimate the concentration of asbestos in the sampled air. (Other aspects of the protocol including counting rules, filter loading, and area of filter examined also affect precision. These are not discussed further here because they can be varied independently of the transfer method. The effect of procedures such as ashing and resuspension that are uniquely associated with indirect transfer method would be included in any overall study of precision.)

Chatfield (1984, 1986) has argued that the spatial distribution of asbestos structures on the filter is closer to random (i.e., follows a Poisson distribution) when an indirect transfer method is used. If structure counts per grid opening are available for Studies 1, 2, 3 and 4, Chatfield's claim can be tested. Efforts are underway to obtain these data. The question may also be addressed experimentally by preparing samples by both techniques and examining the filter in greater detail than is done during routine analysis. A relatively simple statistical design and analysis would be sufficient to detect marked differences and could provide a definite recommendation. A more sophisticated experiment is needed to explore heterogeneity on various spatial scales in order to determine the advisability of preparing more than one portion of the filter or analyzing multiple grids.

Since breakup of structures (resulting in a positive bias) and uneven spatial distribution of structures on the filter (resulting in decreased precision) are claimed to be the major disadvantages of the indirect and direct transfer methods respectively, further research to support or reject these claims would be a valuable and relatively low cost contribution to the continuing discussion over the choice of analytical protocol.